Nanomicelle-enhanced, asymmetric ERED-catalyzed reductions of activated olefins. Applications to 1-pot chemo- and bio-catalysis sequences in water

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Electronic Supplementary Information

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General Information

Silica gel TLC plates (UV 254 indicator, thickness 200 mm standard grade, glass backed and 230-400 mesh from Merck) or Aluminum Oxide 60 F254 polyester basked plates (Sigma-Aldrich, 0.2 mm thick) were used. The developed TLC plate was analyzed by a UV lamp (254 nm). The plates were further analyzed with the use of an aqueous potassium permanganate stain or butanolic vanillin and developed with a heat gun. All commercially available reagents were used without further purification. A 2 wt % TPGS-750-M/H2O solution was prepared by dissolving TPGS-750-M in degassed HPLC grade water. TPGS-750-M1 was made as described previously and is also commercially available. Reagents were purchased from Sigma-Aldrich, Combi-Blocks, Alfa Aesar, or Acros Organics. Flash chromatography was performed using Silicycle Silicaflash® P60 unbonded grade silica. Codex® Ene Reductase Screening kit is commercially available from Codexis. The ¹H and ¹³C NMR were recorded at 25 °C on either a Varian Unity Inova 500 MHz or a Varian Unity Inova 600 MHz spectrometers in CDCl₃ with residual CHCl₃ (¹H = 7.26 ppm, ¹³C = 77.16 ppm) as the internal standard. Chemical shifts are reported in parts per million (ppm). The data presented will be reported as follows; chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, quin = quintet, m = multiplet), coupling constant (if applicable) and integration. HRMS data were recorded on a Waters Micromass LCT TOF ES+ Premier mass spectrometer using ESI ionization. Chiral HPLC data were collected using an Agilent 1220 HPLC. HRMS data were recorded on a Waters Micromass. LCT α-values were measured on a Perkin Elmer Polarimeter 341 in a cuvette (l=10cm) at 589 nm (Na lamp). Concentration c is given in g/100mL.

Preparation of Buffer Solution

Aqueous 1 M stock solutions of potassium phosphate monobasic (A) and potassium phosphate dibasic (B) were prepared. A pH 7 phosphate buffer solution was then prepared by mixing 38.5 mL of solution A with 61.5 mL of solution B. The pH was controlled and adjusted, if needed, with a 1 M solution of NaOH or HCl. The buffer solution was diluted with HPLC grade water to 0.1 M. 2 wt % of TPGS-750-M, as a wax, was dissolved and used as media of the reaction. 4 and 6 wt % of TPGS-750-M in the buffer solution have also been prepared. TPGS-750-M is available from Sigma-Aldrich (catalog #733857 (solution) or #763896 (wax)). Potassium phosphate monobasic and dibasic were purchased from Sigma Aldrich.
To evaluate the impact of TGPS-750-M on the conversion of the model substrate by ENE-reductase, comparative monitoring was performed. In a 5-dr vial, GHD-105 (20 mg), Glucose (2 equiv, 123 mg) and NADP$^+$ (5 mg) were added followed by addition of 10 mL of phosphate buffer at pH 7 (with or without 2 wt % of TPGS-750-M) to make a stock solution. The vial was lightly swirled to allow the components to fully dissolve. Enone (5 mg) and ENE-reductases (10 mg) were added into seven labeled 1-dr equipped with magnetic stir bars. The stock solution (containing GDH-105, glucose, and NADP$^+$ in phosphate buffer, 1 mL) was added to each 1-dr vial. The reactions were stirred at 35 °C for 24 h. After 24 h, the reactions were extracted with MTBE (5x) and concentrated in vacuo. The samples were analyzed by $^1$H NMR to determine the conversion. (2.47 ppm (s) $\rightarrow$ 1.11 ppm (d)). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/i-propanol 99.5:0.5, flow rate 0.5 mL/min) $t_1$ 15.2 min (minor) $t_2$ 16.42 min (major).
Conversion Monitoring in Phosphate Buffer and TPGS-750-M/Phosphate Buffer

To evaluate the impact of TPGS-750-M on the conversion of four different substrates by ENE-reductase, comparative monitoring with and without surfactant in buffer was performed. In a 5-dr vial, GHD-105 (20 mg), Glucose (2 equiv relative to substrate) and NADP+ (5 mg) were added followed by addition of 10 mL of phosphate buffer at pH = 7 (with or without 2 wt % of TPGS-750-M) to make a stock solution (for substrate 4, 4 and 6 wt % of TPGS-750-M in the buffer solution were also prepared). The vial was lightly swirled to allow the components to fully dissolve. In 1-dr vials equipped with a magnetic stir bar, enone (5 mg) and ERED-103 (10 mg) were added. The stock solution (containing GDH-105, glucose, and NADP+ in phosphate buffer, 1 mL) was added to each 1-dr vial. The reactions were stirred at 35 ºC. At each time interval noted, the reactions were extracted with MTBE (5x) and concentrated in vacuo and monitored by ¹H NMR.

Concentration Effect Studies – Table S2

<table>
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<th>Entry</th>
<th>Concentration (M)</th>
<th>Conversion (%)</th>
</tr>
</thead>
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<td>99</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>0.10</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>0.50</td>
<td>24</td>
</tr>
</tbody>
</table>

To evaluate the impact of increased concentration on the activity of the enzyme in 2 wt % of TPGS-750-M in phosphate buffer, reaction monitoring was performed. In a 1-dr vial equipped with a magnetic stir bar, GHD-105 (2 mg), Glucose (2 equiv to enone, 12.3 mg) and NADP+ (0.5 mg), enone (5 mg) and ERED (10 mg) were added followed by addition of 2 wt % of TPGS-750-M in phosphate buffer at pH = 7 to reach desired concentration (poor stirring of components was observed at 0.5 M). The reactions were stirred at 35 ºC for 24 h. After 24 h the organic layers were extracted with MTBE
(5x) and concentrated in vacuo. The samples were analyzed by $^1$H NMR to determine the conversion. (2.47 ppm (s) $\rightarrow$ 1.11 ppm (d)).

**General Procedure for Enantioselective Reduction of Activated Olefins**

To a 5-dr vial equipped with a magnetic stir bar was added olefin (50 mg), ERED-103 (70 mg), GDH-105 (20 mg), glucose (2 equiv to olefin), and NADP$^+$ (5 mg). 5-7 mL (substrate dependent) of 2 wt % of TPGS-750-M in phosphate buffer at pH = 7 was added to the vial. The reaction was set to stir at 35 °C. The reaction was monitored via TLC or $^1$H NMR. Upon completion, the organic layers were extracted with MTBE (5x), dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo. The crude material was purified via flash chromatography to afford the unsaturated alkane. Absolute stereochemistry was confirmed by comparison to commercially available and / or literature referenced enantiopure compounds. All other products were assigned by analogy.

**General Procedure for Reduction of Activated Olefins**

Racemic ketones were synthesized from a modified literature procedure.$^2$ To an oven-dried round bottom flask, equipped with a magnetic stir bar was added enone (1.0 equiv) and triphenylphosphine oxide (1.0 equiv) The flask was evacuated and backfilled with argon (3x). DCE [0.25M] was added to the flask and the mixture was cooled to 0 °C. Trichlorosilane (2.0 equiv) was added dropwise into the reaction mixture at 0 °C. The reaction was warmed to rt and set to stir for 3-7 h. The reaction was monitored via TLC. Upon completion, the reaction mixture was quenched with saturated NaHCO$_3$ solution and filtered through Celite. The organic layer was extracted with DCM (3x), dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo. The crude material was purified via flash chromatography to afford the unsaturated alkane. The resulting alkane was utilized to determine enantiomeric excess.
For compounds 11 and 13, the racemic ketones were synthesized from a modified literature procedure. To a solution of Pd/C (10 wt % 10 mg) in THF (5.0 mL), were added enal or enone (1.50 mmol, 1.0 equiv), H₂SO₄ (100 µL), and triethylsilane (1.1 equiv). The reactions were set to stir for 6 h. Upon completion the mixture was filtered through a pad of alumina with DCM as the eluent. The organic layer was concentrated *in vacuo* and the crude material was purified by flash chromatography.

**1-Pot ERED Reduction, then ADH Reduction**

\[
\text{Ph} \quad \text{O} \quad \text{Ph} \quad \xrightarrow{\text{ERED-103, GDH-105}} \quad \text{O} \quad \text{Ph} \quad \xrightarrow{\text{ADH-101, NAD^+}} \quad \text{O} \quad \text{OH} \quad \xrightarrow{\text{MgSO}_4} \quad \text{O} \quad \text{Ph} 
\]

ADH-101⁴ is commercially available within the enzyme kit EZK-001 from Johnson Matthey. NAD⁺ was purchased from Bioworld. Isopropanol was purchased from VWR. All other commercially available reagents were used without further purification. To a 5-dr vial equipped with a magnetic stir bar was added olefin (50 mg), ERED-103 (70 mg), GDH-105 (20 mg), NADP⁺ (5 mg) and glucose (2 equiv to olefin). 5 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 °C for 24 h. The reaction was monitored via TLC. Upon completion, MgSO₄ (0.8 mg), NAD⁺ (2.6 mg), NADP⁺ (2.4 mg) and ADH-101 (20 mg) were added in succession. i-PrOH (0.6 mL) was added to the reaction mixture. The reaction was stirred at 35 °C for 5 h. The reaction was extracted with EtOAc (5x). The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated *in vacuo*. The crude material was purified via flash chromatography. See analytical data *(vide infra)*, page S15.

**1-Pot Pd-catalyzed Suzuki-Miyaura coupling, ERED Reduction**

\[
\text{Cl} \quad \text{O} \quad \xrightarrow{\text{Pd(OAc)}_2 0.25 \text{ mol\%} \quad \text{N}_2\text{Phos} 0.18 \text{ mol\%} \quad \text{K}_3\text{PO}_4 \quad \text{H}_2\text{O}} \quad \xrightarrow{2 \text{ wt\% TPGS-750M/H}_2\text{O}} \quad \text{O} \quad \xrightarrow{\text{ERED-103, GDH-105}} \quad \text{O} \quad \xrightarrow{\text{MgSO}_4 2 \text{ wt\% TPGS-750-M/}} \quad \text{O} \quad \text{Ph} \quad \xrightarrow{35 \degree \text{C}, 24 \text{ h}} \quad \text{Ph} \quad \text{F} 
\]

To an oven dried 1-dram vile equipped with a stir bar was added Pd(OAc)₂ (2.25 mg, 0.01 mmol) and N₂Phos⁵ (14.8 mg, 0.018 mmol). The vial was capped with a 14/20 rubber septum sealed with Teflon tape. The vial was evacuated and backfilled with argon three times and left under a continuous
flow of argon. Anhydrous toluene (1 mL) was added to the vile to achieve the desired Pd concentration (50 µL of stock solution equates to 1000 ppm loading for a 0.5 mmol reaction). The mixture was set to stir for 15 min. At this point the catalyst is ready and may be added to the reaction mixture.

To an oven dried, 10 mL flask equipped with a magnetic stir bar was charged aryl chloride (0.25 mmol), organoboron (0.38 mmol), and potassium phosphate (0.38 mmol). The vial was fitted with a rubber septum and sealed with Teflon tape. The reaction flask was purged with argon with the use of a vent needle. At this point, a solution of 2 wt % TPGS-750-M in (0.9 mL) followed by the catalyst solution (125 µL) via syringe. The reaction was monitored by GC-MS. Upon completion of the reaction, the pH was adjusted to 7 with a 1 M HCl and, ERED-103 (70 mg), GDH-105 (20 mg), NADP+ (5 mg) and glucose (2 equiv to olefin) were added to the flask. 5 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 ºC for 24 h. Upon completion the reaction was filtered over a pad of Celite and the organics were extracted with MTBE (5x). The organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude material was purified via flash chromatography. See analytical data (vide infra), page S12.

1-Pot ERED Reduction, then Amine Formation utilizing Transaminase ATA

To a 5-dr vial equipped with a magnetic stir bar was added olefin (20 mg, 1.272.10⁻⁴ mol), ERED-103 (40 mg), GDH-105 (8 mg), glucose (46 mg), and NADP+ (2 mg). 4 mL of 2 wt % of TPGS-750-M in phosphate buffer at pH = 7 was added to the vial. The reaction was set to stir at 35 ºC overnight. The reaction was monitored via TLC or ¹H NMR. The concentration was adjusted to [0.01 M] by adding 8.72 mL of a fresh solution* (triethanolamine, [118 mM], pH = 8.5) previously made (see below the protocol). The ATA-256 (100 mg) was then added and the reaction was stirred at 50 °C. Upon completion, the reaction was quenched by adding 5 N NaOH (1.5 mL) to increase reaction pH > 12 and extracted into EtOAc (5 x 10 mL). The organic layers separated by centrifugation were combined and evaporated. To a 2-dr vial containing the product from the second step were added 2 wt % of TPGS-750-M in water (0.9 mL) and sodium carbonate (40 mg, 3 equiv). The reaction was cooled to 0°C and benzyl chloroformate (18 µL, 1 equiv) was added. The reaction was stirred for 20 min at 0 °C and allowed to warm to rt and stirred overnight. The solution is acidified to pH = 2 (0 ºC) and extracted with EtOAc (3
x 1 mL. The organic layers were combined and dried over anhydrous MgSO$_4$ and concentrated in vacuo. The crude material was purified via flash chromatography to afford the desired compound.

*Fresh solution for step 2:*

In a 20 mL flask were added triethanolamine (0.21 g), isopropylamine (1.06 mL), PLP (3.4 mg) and 2 wt % of TPGS-750-M in water (4 mL). The pH was adjusted to 8.5 with HCl (12 M). The volume was brought to 8.72 mL by adding 2 wt % of TPGS-750-M in water. See analytical data (*vide infra*), page S16.

**1-Pot Pd-catalyzed Cyanation, ERED Reduction, then ADH Reduction**

Aryl bromide (1 equiv, 0.2 mmol), Zn(CN)$_2$ (12.9 mg, 0.55 equiv), Xantphos palladacycle (1.7 mg, 0.7 mol %) were added to a 1-dr vial equipped with a magnetic stir bar. The reaction vial was evacuated and backfilled with argon (3x). PMHS (13 µL, 1 equiv) was added under argon followed by dry THF (40 µL, 10 vol %) and 2 wt % TPGS-750-M/H$_2$O (360 µL). The reaction mixture was stirred at 65 ºC overnight. The reaction was monitored via TLC. Upon completion, the reaction mixture was transferred to a 5-dr vial containing ERED-103 (70 mg), GDH-105 (20 mg), NADP$^+$ (5 mg) and glucose (2 equiv to olefin). 5.6 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 ºC for 24 h. The reaction was monitored via TLC. Upon completion, MgSO$_4$ (0.8 mg), NAD$^+$ (2.6 mg), NADP$^+$ (2.4 mg) and ADH-101 (20 mg) were added in succession. i-PrOH (0.6 mL) was added to the reaction mixture. The reaction was stirred at 35 ºC for 5 h. The reaction was extracted with EtOAc three times. The organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$ and concentrated in vacuo. The crude material was purified via flash chromatography. See analytical data (*vide infra*), page S16.
1-Pot ERED Reduction, Pd/C Nitro-reduction, then Acylation

To a 5-ml vial equipped with a magnetic stir bar was added olefin (1.0 equiv, 50 mg), ERED-103 (70 mg), GDH-105 (20 mg), NADP+ (5 mg) and glucose (2 equiv to olefin). 4 mL of 2 wt % TPGS-750-M in phosphate buffer at pH 7 was added to the vial. The reaction was set to stir at 35 °C for 24 h. The reaction was monitored via TLC. Upon completion, 1 M HCl was added to the reaction until the mixture reached pH 2. 10 wt % Pd/C (20 mg) was then added to the reaction mixture. The reaction vessel was purged with H₂ using a balloon and then fitted with another balloon of H₂. The reaction was allowed to stir at rt overnight, being monitored via TLC. Upon completion, the H₂ balloon was removed and acetic anhydride (2.0 equiv) was added to the mixture. The reaction was set to stir for an additional 4 h, monitored via TLC. Upon completion, the reaction was extracted with EtOAc (5x) and the organic layer was washed with brine, dried over anhydrous Na₂SO₄ and concentrated in vacuo. The crude material was purified via flash chromatography. See analytical data (vide infra), page S17.

References

Experimental Data

(S)-3-Methyl-4-phenylbutan-2-one (1): $^1$H NMR (500 MHz, CDCl$_3$) δ 7.31 – 7.27 (m, 2H), 7.24 – 7.19 (m, 1H), 7.19 – 7.14 (m, 2H), 3.02 (dd, $J = 13.6$, 6.8 Hz, 1H), 2.85 (h, $J = 7.1$ Hz, 1H), 2.58 (dd, $J = 13.6$, 7.7 Hz, 1H), 2.10 (s, 3H), 1.11 (d, $J = 7.0$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) δ 212.24, 139.80, 129.05, 128.55, 126.37, 48.94, 39.05, 28.98, 16.36. **Yield:** 88% (3 h) as a colorless oil; 86% ee. **S enantiomer $\alpha_D^{20.0} = +29.8$ (c 0.981 in CHCl$_3$) (ERED-103) R$_f = 0.28$ (10% EtOAc/hexanes).** The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/i-propanol 99.5:0.5, flow rate 0.5 mL/min) t$_1$ 15.2 min (minor) t$_2$ 16.42 min (major).

(S)-3-Methyl-4-(3-phenoxyphenyl)butan-2-one (2): $^1$H NMR (400 MHz, CDCl$_3$) δ 7.37 – 7.30 (m, 2H), 7.24 (t, $J = 7.8$ Hz, 1H), 7.10 (t, $J = 7.4$ Hz, 1H), 6.99 (d, $J = 7.5$ Hz, 2H), 6.90 (d, $J = 7.6$ Hz, 1H), 6.84 (d, $J = 9.5$ Hz, 2H), 2.98 (dd, $J = 13.6$, 6.7 Hz, 1H), 2.81 (h, $J = 7.1$ Hz, 1H), 2.54 (dd, $J = 13.6$, 7.8 Hz, 1H), 2.10 (s, 3H), 1.09 (d, $J = 7.0$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) δ 212.01, 157.38, 157.34, 141.90, 129.87, 129.81, 124.05, 123.34, 119.51, 118.92, 116.87, 48.73, 38.78, 28.98, 16.39. **Yield:** 91% (24 h) as a pale-yellow oil; 93% ee (ERED-103). R$_f$: 0.25 (10% Et$_2$O/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/i-propanol 98:2, flow rate 1 mL/min) t$_1$ 17.4 min (minor) t$_2$ 18.1 min (major). Molecular formula: C$_{17}$H$_{18}$O$_2$ EI-MS [M]$^+$ calcd: 254.1307; found: 277.1207 [M + Na]$^+$
(S)-3-Methyl-4-(4-nitrophenyl)-3-butan-2-one (3): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.14 (d, $J = 8.7$ Hz, 2H), 7.32 (d, $J = 6.7$ Hz, 2H), 3.12 (dd, $J = 13.6$, 7.2 Hz, 1H), 2.85 (p, $J = 7.1$ Hz, 1H), 2.66 (dd, $J = 13.6$, 7.2 Hz, 1H), 2.12 (s, 3H), 1.14 (d, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 210.96, 147.87, 146.75, 129.97, 123.81, 48.50, 38.39, 28.97, 16.72. Yield: 87% as a pale-yellow oil; 60% ee. S enantiomer $\alpha^D_{20.0} = +10.1$ (c 1.05 in CHCl$_3$) (ERED-103). Rf: 0.25 (20% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Lux 5u Cellulose-2 column, hexanes/i-propanol 95:5, flow rate 0.5 mL/min) $t_1$ 19.08 min (minor) $t_2$ 20.20 min (major).

(S)-3-Methyl-4-(2-nitrophenyl)butan-2-one (4): $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.95 (dd, $J = 8.2$, 1.4 Hz, 1H), 7.51 (td, $J = 7.5$, 1.4 Hz, 1H), 7.41 – 7.30 (m, 2H), 3.34 (dd, $J = 13.3$, 6.9 Hz, 1H), 2.97 (h, $J = 7.0$ Hz, 1H), 2.81 (dd, $J = 13.3$, 7.0 Hz, 1H), 2.12 (s, 3H), 1.13 (d, $J = 7.1$ Hz, 3H). $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 211.49, 135.15, 133.40, 133.11, 127.74, 125.17, 47.46, 35.83, 29.13, 16.80. Yield: 87% as a pale-yellow oil; 91% ee (ERED-103). Rf: 0.30 (25% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/i-propanol 90:10, flow rate 0.7 mL/min) $t_1$ 12.79 min (major) $t_2$ 13.85 min (minor). Spectral data matched those previously reported.¹

(S)-4-(4-Bromophenyl)-3-methyl-3-butan-2-one (5): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.39 (d, $J = 8.3$ Hz, 2H), 7.03 (d, $J = 8.4$ Hz, 2H), 2.95 (dd, $J = 13.7$, 7.0 Hz, 1H), 2.80 (p, $J = 7.1$ Hz, 1H), 2.51 (dd, $J = 13.7$, 7.5 Hz, 1H), 2.09 (s, 3H), 1.09 (dd, $J = 7.0$, 1.3 Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 211.74, 138.86, 131.64, 130.81, 120.24, 48.78, 38.29, 29.03, 16.47. **Yield:** 86% as a colorless oil; 78% ee (ERED=103). $R_f$: 0.30 (10% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/i-propanol 95:5, flow rate 0.5 mL/min) $t_1$ 29.94 min (minor) $t_2$ 31.51 min (major). Spectral data matched those previously reported.\(^2\)

(S)-4-(2'-Fluoro-[1,1'-biphenyl]-4-yl)-3-methyl-3-butan-2-one (6): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.49 (m, 2H), 7.44 (m, 1H), 7.31 (m), 7.27 - 7.18 (m, 3H), 7.16 (m, 1H), 3.07 (dd, $J = 13.6$, 6.8 Hz, 1H), 2.89 (d, $J = 7.0$ Hz, 1H), 2.62 (dd, $J = 13.6$, 7.7 Hz, 1H), 2.15 (s, 3H), 1.15 (d, $J = 7.0$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 212.15, 139.37, 130.79, 130.77, 129.17, 128.99, 128.92, 124.48, 124.45, 116.31, 116.12, 48.89, 38.66, 28.98, 16.48. **Yield:** 82% (73% yield in the 1-pot sequence) as a white solid; 99% ee (ERED=103). $R_f$: 0.30 (10% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/i-propanol 98:2, flow rate 1.0 mL/min) $t_1$ 7.23 min (minor) $t_2$ 7.82 min (major). Molecular formula: C$_{17}$H$_{17}$FO EI-MS [M]$^+$ calcd: 256.1263; found: 279.1162 [M + Na]$^+$

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(S)-3-(4-Nitrobenzyl)octan-2-one (7): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.13 (d, $J = 8.7$ Hz, 2H), 7.30 (d, $J = 8.7$ Hz, 2H), 3.02 (dd, $J = 13.4$, 8.6 Hz, 1H), 2.87 – 2.80 (m, 1H), 2.76 (dd, $J = 13.4$, 5.8 Hz, 1H), 2.03 (s, 3H), 1.69 – 1.61 (m, 1H), 1.50 – 1.42 (m, 1H), 1.32 – 1.23 (m, 6H), 0.90 – 0.85 (t, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 211.17, 147.95, 129.87, 123.84, 54.38, 37.17, 31.94, 31.89, 30.22, 26.86, 22.56, 14.10. Yield: 84% as a pale-yellow oil; 93% ee (ERED-103). $R_f$: 0.20 (15% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/$i$-propanol 90:10, flow rate 1 mL/min) $t_1$ 7.29 min (major) $t_2$ 8.13 min (minor). Molecular formula: $C_{15}H_{21}NO_3$ EI-MS [M]$^+$ calcd: 263.1521; found: 263.1523.

(S)-3-(2,4,5-Trifluorobenzyl)undecan-2-one (8): $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 6.95 (ddd, $J = 10.5$, 8.8, 6.9 Hz, 1H), 6.86 (td, $J = 9.7$, 6.6 Hz, 1H), 2.83 – 2.75 (m, 2H), 2.69 – 2.64 (m, 1H), 2.05 (s, 3H), 1.64 – 1.57 (m, 1H), 1.45 – 1.37 (m, 1H), 1.30 – 1.20 (m, 13H), 0.86 (t, $J = 7.0$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 211.32, 119.05, 118.95, 105.69, 105.46, 105.29, 53.01, 31.96, 31.77, 30.14, 29.92, 29.77, 29.48, 29.33, 27.13, 22.78, 14.22. Yield: 65% as a yellow oil; 98% ee (ERED-103). $R_f$: 0.30 (5% Et$_2$O/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/$i$-propanol 98:2, flow rate 1 mL/min) $t_1$ 3.55 min (minor) $t_2$ 3.81 min (major). Molecular formula: $C_{18}H_{25}$F$_3$O EI-MS [M]$^+$ calcd: 314.1858; found: 326.1232 [M + Na]$^+$
Methyl 2-(2,3-dichlorobenzyl)-3-oxobutanoate (9): As a 1:1 mixture with the enol ether. 1H NMR (500 MHz, CDCl$_3$) $\delta$ 7.35 (m, 1H), 7.19 – 7.16 (m, 1H), 7.12 (m, 1H), 3.97 (dd, $J = 8.2, 6.5$ Hz, 1H), 3.71 (s, 3H), 3.35 (dd, $J = 14.0, 6.6$ Hz, 1H), 3.28 (dd, $J = 14.0, 8.2$ Hz, 1H), 2.25 (s, 3H). 13C NMR (126 MHz, CDCl$_3$) $\delta$ 201.90, 169.30, 129.94, 129.35, 128.92, 128.68, 127.42, 58.45, 42.99, 32.83, 28.77. Yield: 92% as a colorless oil (ERED-103). R$_f$: 0.25 (15% EtOAc/hexanes). Molecular formula: C$_{12}$H$_{12}$Cl$_2$O$_3$ EI-MS [M]$^+$ calcd: 274.0163; found: 275.0241 [M + H]$^+$

(1-Nitropropan-2-yl)benzene (10): 1H NMR (600 MHz, CDCl$_3$) $\delta$ 7.30 (t, $J = 7.3$ Hz, 2H), 7.25 (t, $J = 7.4$ Hz, 1H), 7.15 (d, $J = 7.1$ Hz, 2H), 4.77 (h, $J = 6.8$ Hz, 1H), 3.32 (dd, $J = 14.0, 7.4$ Hz, 1H), 3.00 (dd, $J = 14.0, 6.9$ Hz, 1H), 1.53 (d, $J = 6.6$ Hz, 3H). 13C NMR (126 MHz, CDCl$_3$) $\delta$ 135.64, 129.10, 128.94, 127.54, 84.55, 41.29, 18.92. Yield: 91% as a colorless oil (ERED-103) R$_f$: 0.27 (5% EtOAc/hexanes). Spectral data matched those previously reported.$^3$

Ethyl 2-cyano-3-phenyl-2-propanoate (11): 1H NMR (500 MHz, CDCl$_3$) $\delta$ 7.36 (dd, $J = 8.0, 6.4$ Hz, 2H), 7.34 – 7.27 (m, 3H), 4.25 (q, $J = 7.1$ Hz, 2H), 3.73 (dd, $J = 8.4, 5.8$ Hz, 1H), 3.30 (dd, $J = 13.8, 5.8$ Hz, 1H), 3.21 (dd, $J = 13.8, 8.4$ Hz, 1H), 1.28 (t, $J = 7.2$ Hz, 3H). 13C NMR (126 MHz, CDCl$_3$) $\delta$ 165.64, 135.41, 129.15, 128.99, 127.91, 116.27, 63.05, 39.80, 35.89, 14.05. Yield: 96% as a pale-yellow oil (ERED-P1-A04). R$_f$: 0.30 (10% EtOAc/hexanes). Spectral data matched those previously reported.$^4$

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Ethyl (R)-3-(4-chlorophenyl)-3-cyanopropanoate (12): \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.38 (d, \(J = 8.7\) Hz, 2H), 7.33 (d, \(J = 8.5\) Hz, 2H), 4.29 (t, \(J = 7.4\) Hz, 1H), 4.19 (qq, \(J = 7.1, 3.7\) Hz, 2H), 3.01 (dd, \(J = 16.6, 7.8\) Hz, 1H), 2.83 (dd, \(J = 16.6, 7.0\) Hz, 1H), 1.26 (t, \(J = 7.1\) Hz, 3H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 169.03, 134.84, 133.11, 129.62, 128.94, 119.64, 61.74, 40.00, 32.76, 14.22. Yield: 82% as a colorless oil, \(R\) enantiomer \(\alpha_D^{20.0} = -8.1\) (c0.991 in CHCl\(_3\)) 82% ee (ERED-P1-H09). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/i-propanol 98:2, flow rate 1.0 mL/min) \(t_1\) 17.75 min (major) \(t_2\) 19.92 min (minor). Spectral data matched those previously reported.\(^5\)

\[(2R,3S)-3\text{-Methyl-4-}(3\text{-phenoxyphenyl})\text{butan-2-ol (13)}: \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.34 (dd, \(J = 8.6, 7.4\) Hz, 2H), 7.25 (t, \(J = 7.8\) Hz, 1H), 7.11 (t, \(J = 7.4\) Hz, 1H), 7.04 – 6.99 (m, 2H), 6.94 (dt, \(J = 7.7, 1.3\) Hz, 1H), 6.88 – 6.83 (m, 2H), 3.70 (p, \(J = 6.2\) Hz, 1H), 2.86 (d, \(J = 13.4, 4.9\) Hz, 1H), 2.34 (dd, \(J = 13.4, 9.4\) Hz, 1H), 1.86 – 1.75 (m, 1H), 1.49 (s, 1H), 1.21 (d, \(J = 6.3\) Hz, 3H), 0.85 (d, \(J = 6.8\) Hz, 3H). \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 157.23, 143.32, 129.84, 129.59, 124.35, 123.19, 119.91, 118.84, 116.52, 71.51, 42.27, 39.07, 20.00, 14.78. Yield: 82% as a colorless oil; 77% ee, 56% de (ERED-103, ADH-101). The enantioselectivity was determined by HPLC analysis (Lux 5u Cellulose-2 column, hexanes/i-propanol 99:1, flow rate 0.5 mL/min) \(t_1\) 16.94 min (minor) \(t_2\) 19.93 min (major). Molecular formula: C\(_{17}\)H\(_{18}\)O\(_2\) El-MS [M]\(^+\) calcd: 254.1307; found: 236.1210 [M – H\(_2\)O]\(^+\)

Benzyl (2R,3S)-3-methyl-4-phenylbutan-2-yl)carbamate (14): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.43 – 7.29 (m, 5H), 7.29 – 7.24, 7.23 (m, 2H) – 7.04 (m, 3H), 5.11 (s, 2H), 4.61 (s, 1H), 3.83 (s, 1H), 2.76 (dd, $J = 13.4$, 5.4 Hz, 1H), 2.32 (dd, $J = 13.4$, 9.1 Hz, 1H), 1.89 (s, 1H), 1.15 (d, $J = 6.7$ Hz, 3H), 0.82 (d, $J = 6.8$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 155.9, 140.8, 136.7, 129.1 (2C), 128.6, (2C) 128.3 (3C), 128.2 (2C), 125.9, 66.6, 50.5, 40.3, 39.6, 18.4, 14.5. Yield: 63% as a yellow oil; 79% ee; 78% de (ERED-103, ATA-256. The enantioselectivity was determined by HPLC analysis (Chiracel AD-H column, hexanes/i-propanol 98:2, flow rate 0.7 mL/min) t$_1$ 43.14 min (major) t$_2$ 55.52 min (minor) HRMS (m/z): [M+Na]$^+$ calcd. for C$_{19}$H$_{23}$NO$_2$Na, 320.1627; found, 320.1629

4-((2S,3R)-3-Hydroxy-2-methylbutyl)benzonitrile (15) $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.56 (d, $J = 7.9$ Hz, 2H), 7.27 (d, $J = 8.0$ Hz, 2H), 3.65 (p, $J = 6.2$ Hz, 1H), 2.98 (dd, $J = 13.4$, 4.5 Hz, 1H), 2.39 (dd, $J = 13.4$, 9.6 Hz, 1H), 1.78 (dtd, $J = 12.4$, 6.5, 3.3 Hz, 1H), 1.21 (d, $J = 6.3$ Hz, 3H), 0.79 (d, $J = 6.8$ Hz, 3H); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 147.15, 132.19, 132.15, 130.10, 130.05, 119.24, 109.75, 71.33, 42.17, 39.17, 20.45, 14.89. Yield: 54% as a light-yellow oil; >99% ee; 12:88 dr (ERED-103, ADH-101). R$_f$ 0.31 (35% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OD-H column, hexanes/i-propanol 98:2, flow rate 0.7 mL/min) t$_1$ 52.31 min (minor) t$_2$ 54.02 min (major). Molecular formula: C$_{12}$H$_{15}$NO El-MS [M]$^+$ calcd: 189.1154; found: 171.1053 [M – H$_2$O]$^+$
1-((2S,3S)-2,3-Dimethyl-3,4-dihydroquinolin-1(2H)-yl)ethan-1-one (16): syn : anti = 90:10; For syn isomer: \( ^1H \) NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.13 (m, 4H), 2.92 (dd, \( J = 17.5, 6.7 \) Hz, 1H), 2.44 (dd, \( J = 17.5, 11.6 \) Hz, 1H), 2.24 (s, 3H), 2.21 – 2.15 (m, 1H), 1.14 (t, \( J = 6.9 \) Hz, 1H), 1.01 (d, \( J = 6.8 \) Hz, 3H), 0.92 (d, \( J = 6.9 \) Hz, 3H). \( ^{13}C \) NMR (126 MHz, CDCl\(_3\)) \( \delta \) 170.08, 129.19, 125.86, 125.61, 124.88, 32.37, 32.07, 29.85, 23.75, 19.75, 18.76. Yield: 62% as a yellow oil; >99% ee; 72:28 dr (ERED-103). \( R_f \): 0.30 (25% EtOAc/hexanes). The enantioselectivity was determined by HPLC analysis (Chiracel OJ-H column, hexanes/i-propanol 95:5, flow rate 0.8 mL/min) \( t_1 \) 7.04 min (minor) \( t_2 \) 8.34 min (major). Molecular formula: \( \text{C}_{13}\text{H}_{17}\text{NO} \) EI-MS [M\(^+\)] calcd: 203.1310; found: 204.1388 [M + H\(^+\)]
$^1$H, $^{13}$C Spectra of Products

![Chemical Structure](image)
HPLC Data of Compounds

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The retention times of the racemate and the product almost align. It is slightly shifted by 0.8 mins.
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The retention times of the racemate and the product almost align. It is slightly shifted by 1 min.
The small peak around 7 min is not an enantiomer. It is a small impurity that has an area of <1%.
The retention times of the racemate and the product almost align. It is slightly shifted by <0.8 mins.
The small peak around 3.5 min is not an enantiomer. It is small impurity that has an area of <1%.
For compound 13, the HPLC analysis revealed an ee of 77% ((88.5504-11.4496)/100). In the top HPLC of the racemic material, the peak at 18.8 min represents two peaks that are a combination of the ratio of the peak at 17.7 and 21.0. In the bottom HPLC assessing ee, only two peaks are present that assumes the second step (the asymmetric ketone reduction with KRED) is ca. 100% ee, hence, the ratio of these two peaks equals the ee of the ERED step.
The dr was determined by NMR spectra. The relative integrations of the two doublet of doublets, corresponding to the protons resulting from the asymmetric ERED-catalyzed reductions, reveal the diastereoisomeric ratio obtained. Moreover, the NMR experiments and the signal ratio is directly proportional to the diastereomeric ratio (dr). Thus, the integration of protons at 2.86 ppm (0.78/1) and 2.80 (0.22/1) (one from each diastereomer) gives a dr of 78/22 (56% de).
The HPLC analysis reveals an ee of 79% (89.3258-10.6732)/100. The HPLC for racemic material (above) shows 4 peaks of essentially equal area % representing the enantiomers of the two pairs of diastereomers. In the trace below from the reaction sequence, only two peaks are present, and since both outcomes from each enzymatic process is known (as shown), these must represent the ee associated with this particular diastereomer.

The dr was determined by NMR spectra. The relative integrations of the two doublet of doublets, corresponding to the proton resulting from the asymmetric ERED-catalyzed reductions of activated olefins reveal the diastereoisomeric ratio obtained. Moreover, the signal ratio from the NMR experiments is directly proportional to the diastereomeric ratio (dr). So, the integration of protons at 2.80 (0.11/1) and 2.76 ppm (0.89/1) (one from each diastereomer) enzyme gives a dr of 89/11 (78% de).
The HPLC data of the racemic material (top HPLC trace) shows both diastereomers in roughly a 1.2:1 ratio. The product (lower HPLC trace) shows three of the four enantiomers of the two possible diastereomers. However, the first two peaks at 49.8 and 52.3 min correspond to one of the two diastereomers, while the largest peak is the only one observed for the major isomer. Hence, the ee is >99%.
Supporting this assignment, the UV spectra, with peaks at 49.81 min and 52.31 min represent enantiomers. The major peak at 54.02 min in the product (lower) HPLC shows no presence of enantiomers. Thus, we can conclude in addition to the ee being >99%, the dr is the ratio between the sum of the first two peaks and the third peak (12:88).
According to the (top) HPLC for the racemic mixture, the peak at 6.93 min has two peaks embedded in this one peak (they are very challenging to separate). The left half of this peak is one enantiomer matched with the peak at 8.31 min since they have the same UV spectra. The right half of the peak at 6.93 min corresponds to the enantiomer which is matched with the peak at 10.65 min. The HPLC data of the product (bottom trace) shows two peaks (the peak at ~11.44 is an impurity). The UV spectra of the peak at 7.04 min and the peak at 8.34 min are not identical indicating that each peak represents an enantiomerically pure component of each diastereomeric pair. Thus, with no enantiomeric peaks visible, these HPLC traces indicate that the ee’s are >99% for each, while the dr is as shown (28:72).